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Research Article

Evaluation of The Therapeutic Potential of Aqueous Extract Of *Nephrolepis biserrata* on Testosterone Propionate-Induced Benign Prostatic Hyperplasia: Modulation of Oxidative Stress Enzymes And Inflammatory Biomarkers

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Abstract

The prostate gland is located just below the bladder in men and surrounds the top portion of the tube that drains urine from the bladder (urethra). The prostate's primary function is to produce the fluid that nourishes and transports sperm (seminal fluid). Benign prostatic hyperplasia (BPH) can be defined as uncontrolled, albeit non-malignant proliferation of prostatic glandular and stromal cells. It is a chronic age- related condition affecting almost 3 out of 4 men in their sixties. Benign prostatic hyperplasia (BPH) is the most common neoplasm and a significant cause of urinary symptoms in adult males. Prostate and testicular damage associated with testosterone propionate exposure has been reported to involve prostate disruption and testicular damage which results in a sequalae of physiologic and pathologic responses. Whether *Nephrolepis biserrata*, a widely used herb for a wide range of disease treatment can attenuate the adverse effect of testosterone propionate exposure remains poorly understood. Hence, this study investigated the effect of *Nephrolepis biserrata* on testosterone propionate induced benign prostatic hyperplasia in male Wistar rat. Following 14 days acclimatization, rats were induced with 8 mg/kg b.wt of testosterone propionate through subcutaneous administration for 11, 22 and 33 days respectively, thereafter treated with 100 mg/kg b.wt, 200 mg/kg b.wt and 300mg/kg b.wt of *Nephrolepis biserrata* aqueous leaf extract orally for 11, 22 and 33 days respectively. At the end of 11, 22 and 33 days of experimentation, animals were euthanized, blood and tissues (prostate and testis) were collected for oxidative stress makers and inflammatory markers. The results showed that *Nephrolepis biserrata* treatment improves anti-inflammatory and oxidative stress functions. *Nephrolepis biserrata* significantly attenuated testosterone propionate induced prostate and testicular damage by reducing inflammation of the prostate and testicular tissues. The level of IL-6 was reduced from $56.7 \pm 8.82 pg/ml$, $2.3 \pm 5.04 pg/ml$ and $58.0 \pm 3.00 pg/ml$ for the negative control to $2.68 \pm 0.32 pg/ml$, $11.3 \pm 2.40 pg/ml$ and $7.10 \pm 1.40 pg/ml$ for the groups treated with 300mg/kg b.wt of the extract. *Nephrolepis biserrata* treated groups also showed attenuation of the oxidative stress makers (MDA, SOD, CAT and GPx) with highly elevated MDA concentration reduced and the activities of the stress enzymes increased. This study suggests that *Nephrolepis biserrata* exhibit therapeutic potential against testosterone propionate induced benign prostatic hyperplasia by enhancing anti-inflammatory functions and antioxidative potential.

Abbreviation: SOD-Superoxide Dismultase; MDA-Malondialdehyde; CAT-Catalase; GPX-Glutathione Peroxidase; IL-6-InterLeukin 6; PSA-Prostate Specific Antigen; TNF α-Tumor Necrosis Factor alpha; 5 AR- 5 Alpha Reductase; CRP- C Reactive Protein; TP- Testosterone propionate.

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1. Introduction

The prostate gland is located just below the bladder in men and surrounds the top portion of the tube that drains urine from the bladder (urethra). The prostate's primary function is to produce the fluid that nourishes and transports sperm (seminal fluid). The prostate's most important function is the production of a fluid that, together with sperm cells from the testicles and fluids from other glands, makes up semen. The muscles of the prostate also ensure that the semen is forcefully pressed into the urethra and then expelled outwards during ejaculation. Benign prostatic hyperplasia (BPH) can be defined as uncontrolled, albeit non-malignant proliferation of prostatic glandular and stromal cells [1]. It is a chronic age- related condition affecting almost 3 out of 4 men in their sixties [2]. Benign prostatic hyperplasia (BPH) is the most common neoplasm and a significant cause of urinary symptoms in adult males [3]. Enlargement of the prostate occurs with age leading to bladder out-let obstruction, which manifests with symptoms of impaired urine voiding and/or storage referred to as lower urinary tract symptoms (LUTS). Although BPH is not life threatening, its clinical manifestation as LUTS reduces the patient's quality of life (QOL) [4]. The prevalence of BPH varies and depends on the criteria as well as research settings. Several community-based epidemiological studies have documented the prevalence of BPH ranging from 30 to 50% and 18.1 to 25.3% in hospital-based and community-based settings respectively [5, 6]. However, such studies are relatively scarce in sub-Saharan Africa where almost all the existing reports are hospital-based settings. According to [5], cultural and linguistic differences accounts for differences in the reported prevalence of LUTS and BPH among countries. As the population of aging men increases, BPH has become an important topic of public health concern. Community-based studies in Nigeria utilized the international prostrate symptom score (IPSS) as the only tool to determine the prevalence of BPH [7]. Another study conducted amongst Ghanaian men utilized IPSS and prostatic enlargement by digital rectal examination (DRE) [8]. The pathophysiology of this disease remains poorly understood. Numerous studies have reported oxidative stress, suppression of apoptosis and proliferation surge as crucial mechanism for the development and progression of BPH [9]. Moreover, oxidative stress can activate transcription factor NF-kB which in turn regulates inflammatory and cellular proliferation pathways [10]. The suppression of cell apoptosis affects a rise in the total number of both stromal and epithelial cells, and this has been frequently associated with the development of BPH. Benign prostatic hyperplasia (BPH) is one of the most prevalent urological tract conditions in men over 50 years of age [11]. The estimated prevalence of diagnosed prostatitis is 9%, presenting an overall lifetime prevalence of 14% [12]. BPH is a disease affecting men of all ethnicities.

The emerging attractive strategy for treating BPH is targeting IGF-1/IGF-IR axis genes in the prostatic stoma [13]. Protection of normal tissue during acute and chronic exposure to insults or disease signaling stimuli is still a matter of utmost concern. Several agents, natural or synthetic, have been evaluated for their chemo protective potentials. However, doubts about the efficacy and safety of some of the synthetic agents at their effective chemo protective concentrations have underscored the search for novel, safer, more effective and affordable remedies. *Nephrolepis biserrata*L. (Nephrolepidaceae) is commonly known as Ebelelako among the Urhobo speaking people of Delta state, Nigeria. It goes by the common name buckler fern. It is a perennial fern with green compound leaves (fronds). It is mostly found in shaded and wet places such as river banks and swamps [14]. The majority of the species have stout, slowly creeping rootstocks that form a crown with a vase-shaped ring of fronds. They have spherical sari with a pectate indusium and prominent scales on the stripes. The tip of young shoots of *N. biserrata* is used as a vegetable by the local people in Sabah, Malaysia [15]. Traditionally, N. biserrata is prescribed for the treatment of various diseases. The plant is used to avoid miscarriage, for fetus development and treatment of various microbial infections. [16]. Malan and Neuba (2011) have reported its deployment for the treatment of stomach pain, bleeding and wound healing. Among the local people of Sarawak, Malaysia, it's used for the treatment of skin disorders [17]. Its other pharmacological properties such as antioxidants function and liver protective functions have been reported [18].

2. Materials and Methods

Reagents and Chemicals

Oxidative stress enzymes (CAT, SOD, GPx) and MDA as well as inflammatory biomarkers (IL-6, TNF-α, 5α-reductase and PSA) ELISA kits were purchased from University of Port Harcourt Teaching Hospital through Elabsience representative (manufactured by Elabsience). Testosterone propionate (25mg/ml), Finasteride (5mg/tablet) and Doxazosin (4mg/tablet) were purchased from Nafole Pharmacy, Port Harcourt, Rivers State.

Collection and identification of *Nephrolepis biserrata*

The fresh leaves of *Nephrolepis biserrata* (NB) were collected from Choba River bank, in Choba community, Obio/Akpor Local Government Area of Rivers State, Nigeria. The plant sample was identified and authenticated by Dr. Ekeke Chimezie at the Herbarium Unit of the Department of Plants Science and Biotechnology (PSB), University of Port Harcourt. The sample was registered with voucher number UPH/P/393 and was deposited in the herbarium.

Plant Extraction

The aqueous extract was prepared according to the method described by Sofowora (1993). The fresh leaves of *Nephrolepis biserrata* was washed in a running tap water and was allowed to air dried it was then blended into powder form. 25g of the powder was macerated in 100ml of deionized water for 24 hours using mechanical agitation at room temperature. The suspension was filtered using Whatman filter paper and concentrated in a water bath at approximately 550C. The crude extract was obtained and stored in the refrigerator at 40C.

Phytochemical Screening of the Plant

The fresh leaves of the plant were subjected to phytochemical analysis according to the method outlined by [19, 20]. The phytochemical analysis was done to detect the presence of secondary metabolites, such as alkaloids, glycoside, saponins, tannins, flavonoids, phenolic compounds, steroids, polyphenols and cardiac glycosides.

Study Animals

A total of eighty male Wistar rats weighing between 200-250g and above 10 weeks of age were obtained from the animal house, Faculty of Basic Medical Sciences, University of Port Harcourt. The animals were kept and maintained in polythene cages with typical laboratory conditions. The animals were acclimatized for two weeks.

Experimental Design

Following acclimatization for 14 days, animals were grouped into 16 groups of 5 animals each and treated as follows: Group 1: distilled water and normal rat chow for 33 days
Group 2: 8mg/kg b. wt of Testosterone propionate (TP) for 11 days
Group 3: 8mg/kg b. wt of TP for 22 days
Group 4: 8 mg/kg b. wt of TP for 33 days
Group 5: TP induced + 5mg/kg b. wt of
Finasteride+1 mg/kg b. wt Doxazosin for 11 days
Group 6: TP induced + 5 mg/kg b. wt Finasteride + 1 mg/kg b. wt Doxazosin for 22 days
Group 7: TP induced + 5mg/kg b. wt Finasteride + 1 mg/kg b. wt Doxazosin for 33 days
Group 8: TP induced + 100mg/kg b. wt of N. biserrata for 11 days
Group 9: TP induced + 100mg/kg b. wt of N. biserrata for 22 days
Group 10: TP induced + 100mg/kg b. wt of N. biserrata for 33 days
Group 11: TP induced + 200mg/kg b. wt of N. biserrata for 11 days
Group 12: TP induced + 200 mg/kg b. wt of N. biserrata for 22 days
Group 13: TP induced + 200 mg/kg b. wt of N. biserrata for 33 days
Group 14: TP induced + 300mg/kg b. wt of N. biserrata for 11 days
Group 15: TP induced + 300mg/kg b. wt of N. biserrata for 22 days
Group 16: TP induced + 300 mg/ kg b. wt of N. biserrata for 33 days
At the end of the treatment period, animals were sedated with chloroform. Blood were collected through cardiac puncture into plane bottles for serum biochemical parameters analysis.

Biochemical Analysis

Inflammatory markers: C reactive protein (CRP), Interleukin-6(IL-6), 5-α reductase (5α-R), prostate specific antigen (PSA) and oxidative stress markers superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), glutathione peroxidase (GPx) were determined using blood serum and tissue homogenates. The levels of these markers were estimated using ELISA kits (Elabsience Biotechnology Co., Ltd., China) by standard laboratory procedure using their respective kits manufacturer's instructions.

Statistical Evaluation

The data were subjected to inferential statistics using one-way analysis of variance (ANOVA) and multiple comparison tests was done using Tukey Post Hoc Test in GraphPad Prism 8.0 (GraphPad Software, San Diego, CA). The significance of the data was assessed using a p-value of less than 0.05 and reported as Mean $\pm$Standard Error of Mean (SEM).

3. Results

Qualitative Phytochemical Screening of the Leaves of *Nephrolepis biserrata*

The qualitative phytochemical screening of the leaves of *Nephrolepis biserrata* shows the presence of the following secondary metabolites-Alkaloids, flavonoids, saponins, phenols, steroids, tannins and terpenoids Table 1.

Table 1: Qualitative Phytochemical Composition of the Leaves of *Nephrolepis biserrata*.

Phytochemical	Status
Alkaloids	+
Flavonoids	+
Saponins	+
Phenols	+
Steroids	+
Tannins	+
Terpenoids	+

Effect of Aqueous Extract of *Nephrolepis biserrata* on Inflammatory Markers

Following treatment with testosterone propionate, there was a statistically significant ($p<0.05$) increase in level of PSA for the negative control groups. Similar observation was made for the levels of TNF-α, IL-6 and CRP upon exposure to testosterone propionate with significant ($p<0.05$) increase in the levels of TNF-α, IL-6 and CRP in the negative control groups (5.16 $\pm$0.89 pg/ml to 9.25 $\pm$0.56 pg/ml,

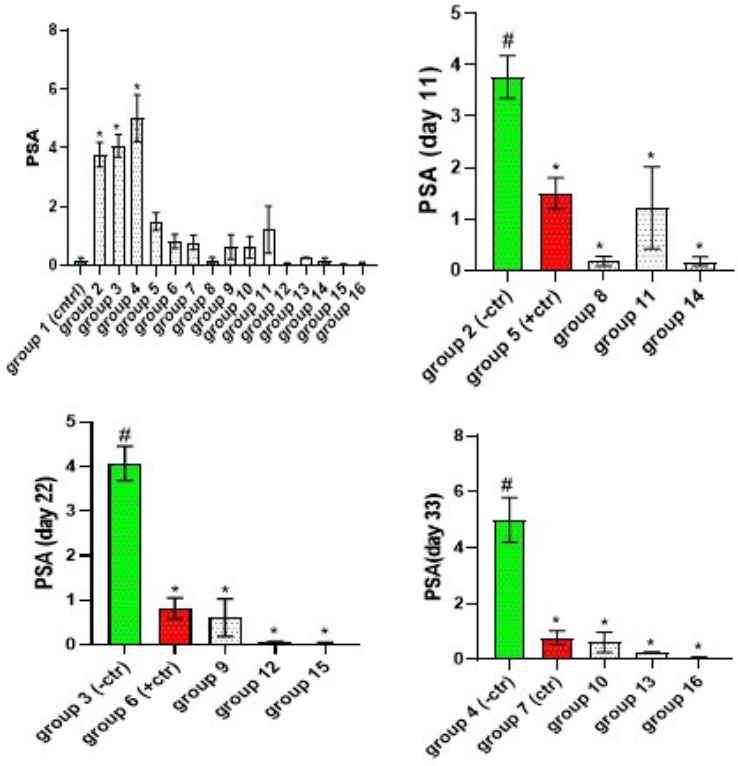

Figure 1: Effect of Aquoeus Extract of *N.biserrata* on PSA Level

13.5 ± 0.72 pg/ml, 16.6 ± 1.33 pg/ml; 14.3 ± 5.36 pg/ml to 56.7 ± 8.82pg/ml, 52.3 ± 5.04pg/ml, 58.0 ± 3.00 pg/ml; and 4.20 ± 0.65mg/L to 6.47 ± 0.30 mg/L, 7.07 ± 0.47 mg/L, 6.67 ± 0.67mg/L respectively). There was also a statistically significant increase ($p < 0.05$) in the activity of 5α-reductase upon exposure to testosterone propionate (8mg/kg b. wt daily). However administration of the plant extract at doses of 100mg/kg b.wt, 200mg/kg b.wt and 300mg/kg b.wt significantly ($p < 0.05$) ameliorated the inflammatory response by reducing the levels of PSA, TNF-α, IL-6 and CRP and the activity of 5α-reductase in the extract treated group Figure 1-5.

Effect of Aqueous Extract of *Nephrolepis biserrata* on Oxidative Stress Markers of Testis and Prostate Tissues

Figure 6-9 show the effect of aqueous extract of *Nephrolepis biserrata* on the oxidative stress makers of testes tissues. The MDA concentration for the negative control groups were 10.2 ± 1.55 nmol/mg protein, 11.1 ± 2.33 nmol/mg protein and 16.3 ± 4.10 mg protein for 11days, 22days and 33days respectively. This was a significant ($p < 0.05$) increase when compared to the normal control (.23 ± 0.83 nmol/mg protein). The negative control groups which received 8mg/kg b. wt of testosterone-propionate only showed a significant ($p < 0.05$) decrease in the activities of catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD). Figures 10-13 show the effect of aqueous extract of *Nephrolepis biserrata* on the oxidative stress makers of prostate tissues. The MDA concentration of the negative control groups were 25.2 ± 2.13 nmol/mg protein, 22.7 ± 5.44 nmol/mg protein and 23.7 ± 5.78 nmol/mg protein for 11days, 22days and 33days respectively. This was a significant ($p < 0.05$) increase when compared to the normal control (7.27 ± 0.68 nmol/mg protein). The negative control groups which received 8mg/kg b. wt of testosterone propionate only showed a significant ($p < 0.05$) decrease in the activities of catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD).

4. Discussion

Benign prostatic hyperplasia is a progressive health condition caused by the enlargement of the prostrate organ. It is usually accompanied by bladder outlet obstruction and Lower Urinary Tract Symptoms (LUTS) [21]. The burden of clinical BPH is very high amongst Nigerian men and the prevalence increases with age. In the study, prevalence of clinical benign prostrate hyperplasia amongst community-dwelling men in South-western Nigerian rural setting, an overall prevalence of BPH of 23.7% or 237 per 1000 men was reported and it also stated that the age-adjusted prevalence increases with increasing age [22]. Other reports have asserted a prevalence of approximately 50% in men in between 50-60 years and greater than 83% in men older than 80 years old [23]. The major treatment options are surgical and pharmacological approaches. Albeit the drawback to these include both surgical complications and the adverse effects associated with the pharmacological agents [24, 25]. An emerging alternative therapy in BPH management is Phyto therapeutics. Many plants have demonstrated anti-BPH effects [26]. In this current study, *Nephrolepis biserrata* is assayed for its therapeutic potency against BPH.

Inflammation is a critical factor in the progression of BPH and various cytokines have been involved in the inflammatory action [27]. In the current study, exposure to testosterone propionate significantly resulted in inflammatory reactions manifesting in the significant release of IL-6, PSA, TNF-α, 5α-R and CRP when compared to the normal control. However, treatment with the plant extract (100mg/kg b. wt, 200mg/kg b. wt and 300mg/kg b. wt) for 11,22 and 33 days significantly abated these inflammatory reactions by suppressing the release of IL-6, PSA, 5α-R and CRP comparatively to the testosterone propionate only treated groups. Testosterone and dihydrotestosterone (DHT)

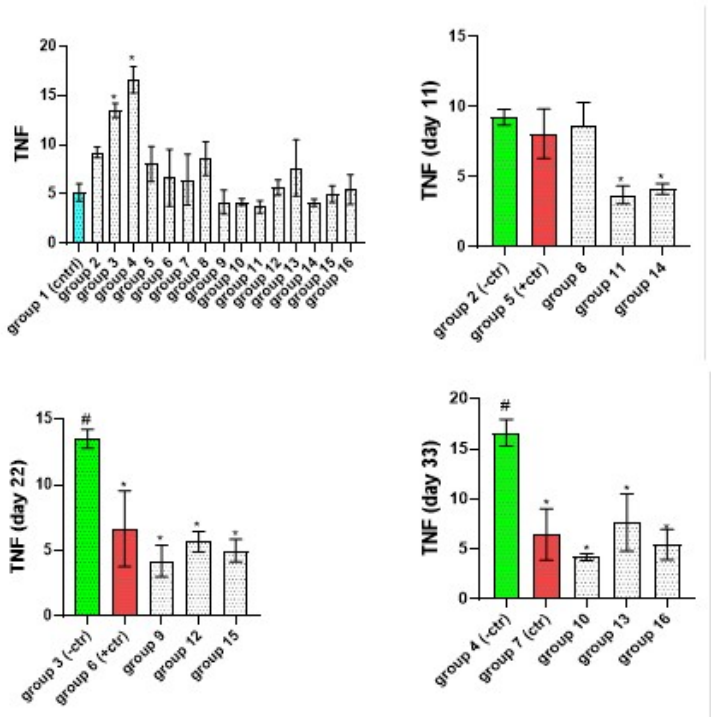

Figure 2: Effect of Aqueous Extract of *N.biserrata* on TNF-α level

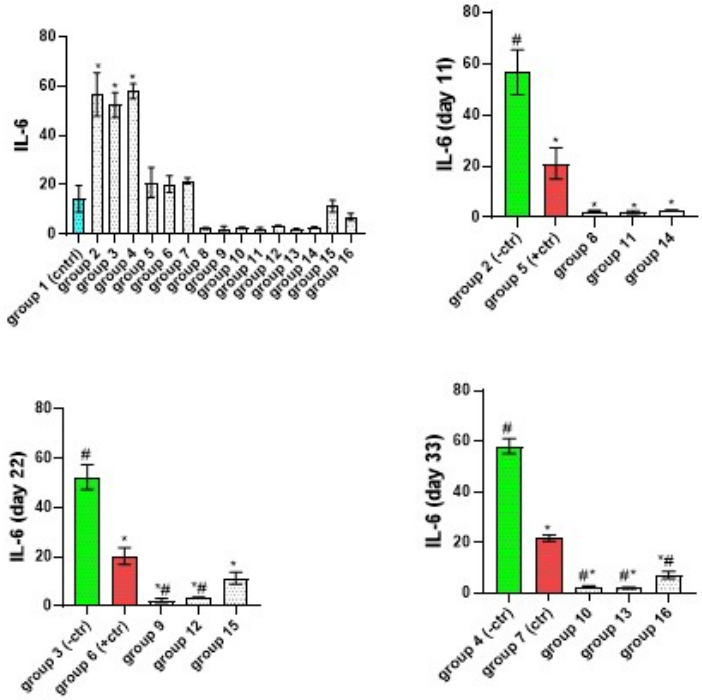

Figure 3: Effect of Aqueous Extract of *N.biserrata* on IL-6 Level

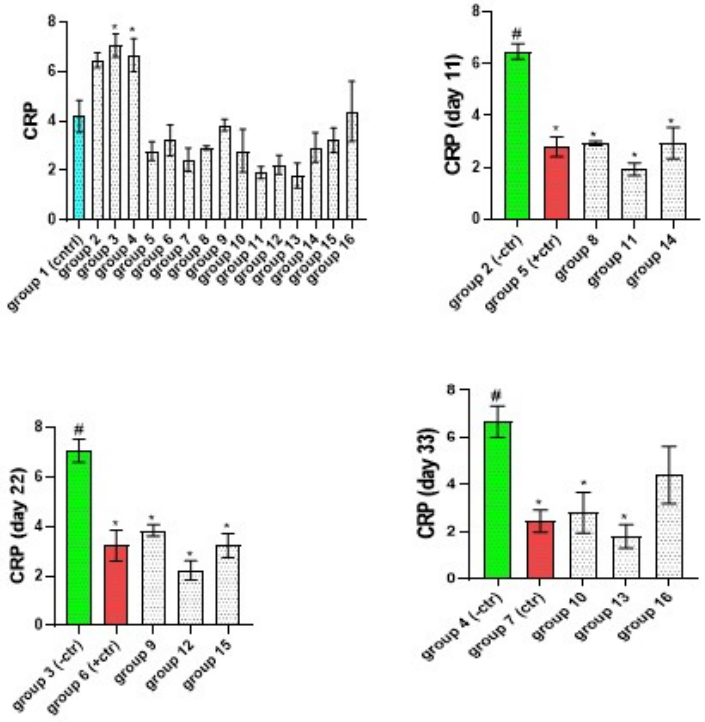

Figure 4: Effect of Aqueous Extract of *N.biserrata* on CRP Level

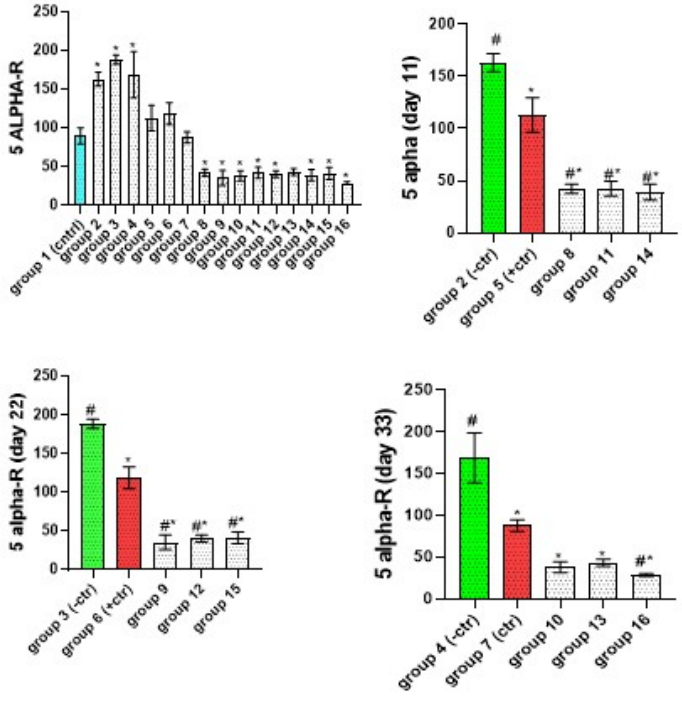

Figure 5: Effect of Aqueous Extract of N. biserrata on 5α-R Activity

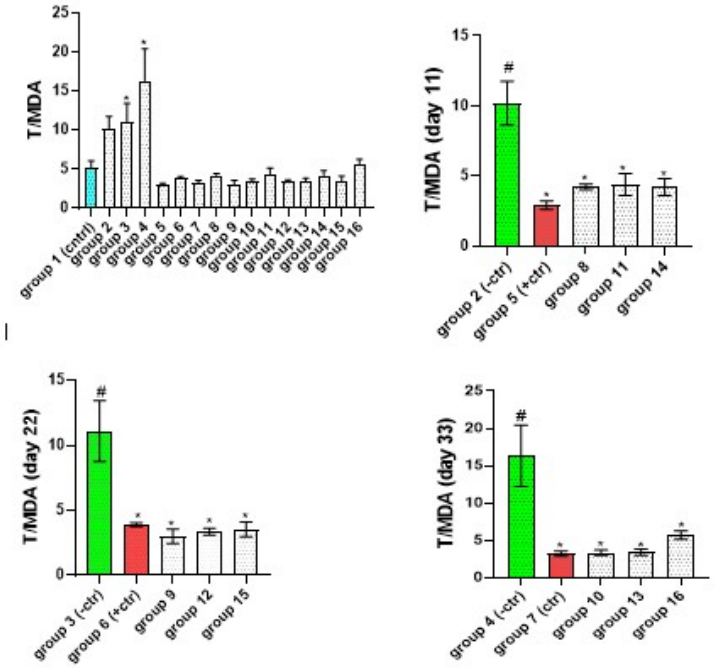

Figure 6: Effect of Aqueous Extract of *N.biserrata* on MDA Concentration of Testis tissue

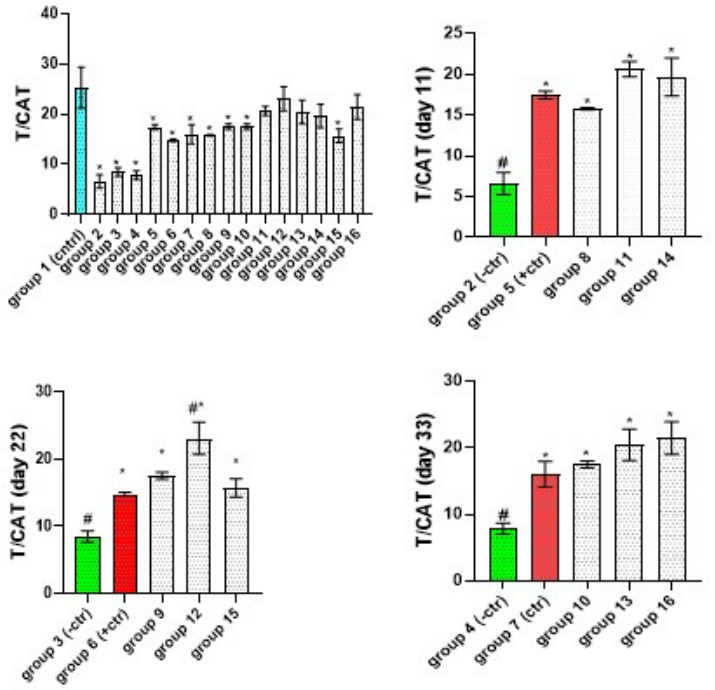

Figure 7: Effect of Aqueous Extract of *N.biserrata* on CAT activity of Testis tissue

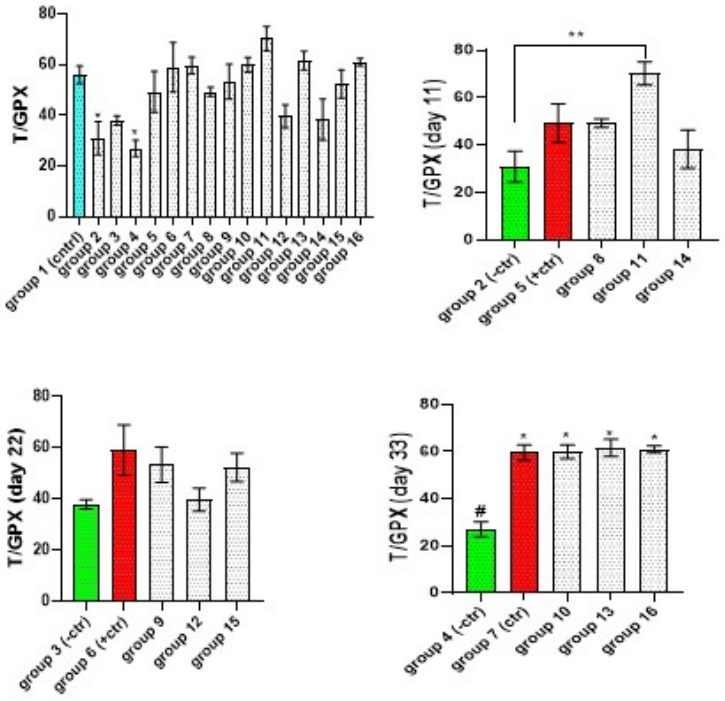

Figure 8: Effect of Aqueous Extract of *N.biserrata* on GPx activity of Testis tissue

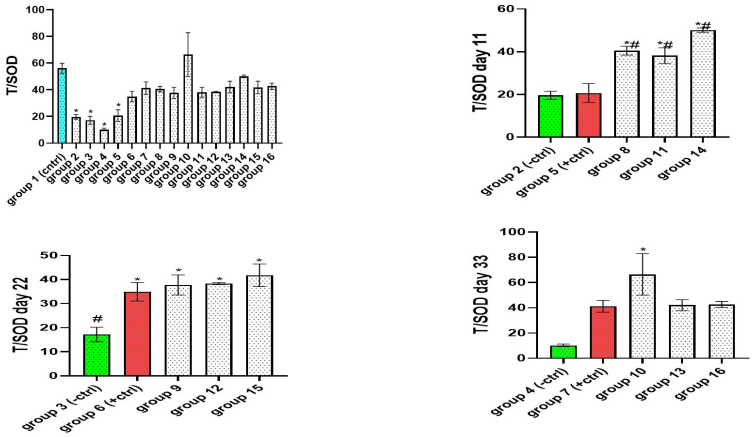

Figure 9: Effect of Aqueous Extract of *N.biserrata* on SOD activity of Testis tissue

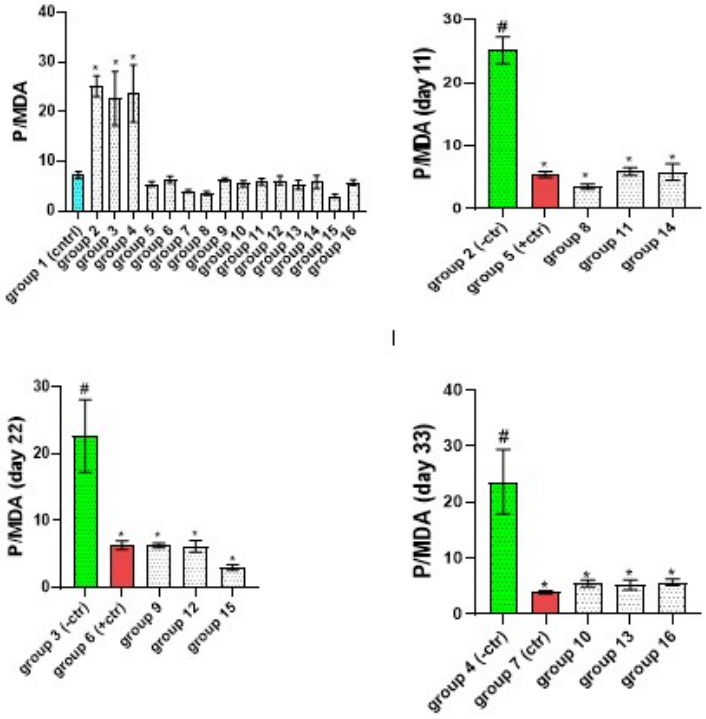

Figure 10: Effect of Aqueous Extract of *N.biserrata* on MDA Concentration of Prostate tissue

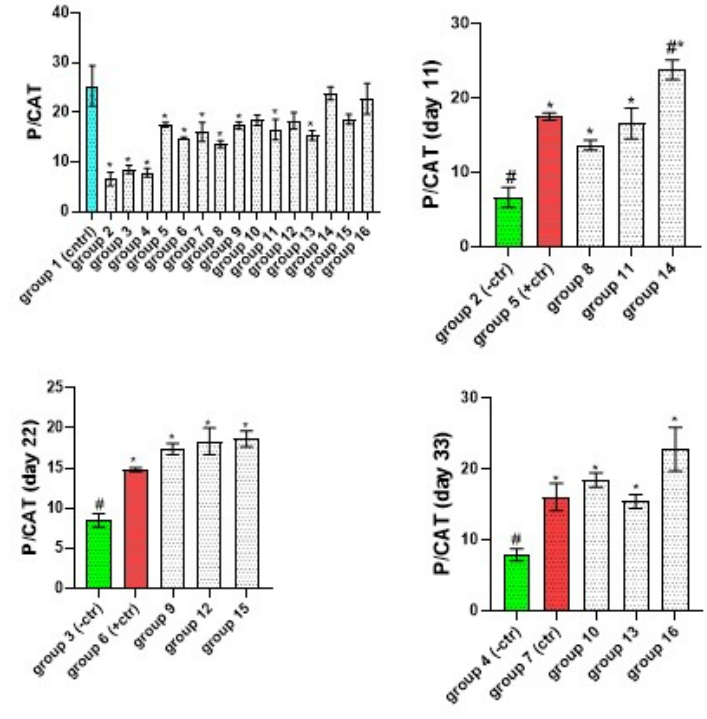

Figure 11: Effect of Aqueous Extract of N. biserrata on CAT Concentration of Prostate tissue

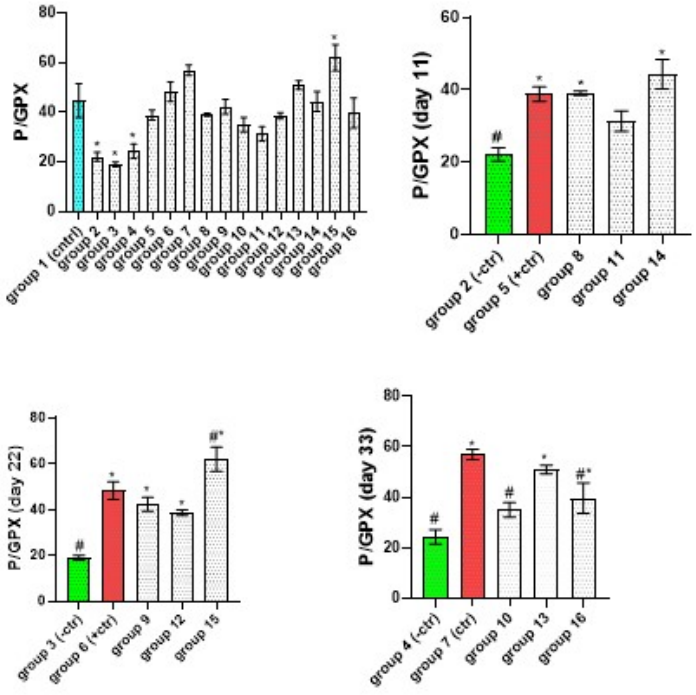

Figure 12: Effect of Aqueous Extract of *N.biserrata* on GPx activity of Prostate tissue

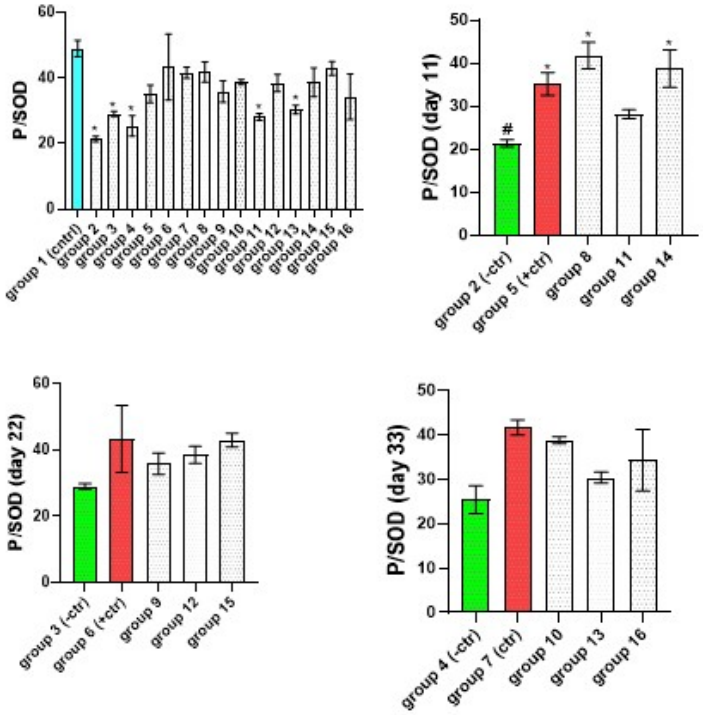

Figure 13: Effect of Aqueous Extract of *N.biserrata* on SOD activity of Prostate tissue

play key roles in the development of BPH [28]. DHT is an important androgen in sexual and reproductive function regulation in males. It has a high affinity for the androgen receptor [29]. 5 α- reductase, catalyzes the conversion of testosterone to dihydrotestosterone [30]. Inhibition of 5α- reductase is a major mechanism of BPH treatment as evidenced by the α-blockers class of drugs such as Finasteride. In this study it was observed that the activity of the enzyme, 5α- reductase was reduced in the extract treated groups, hence hindering the conversion of testosterone to dihydrotestosterone and subsequent progression of BPH. The prostrate is considered an immune-competent organ and it would undergo inflammation after antigen stimulation [31]. Other reports have asserted that inflammation-induced damage of prostatic tissue represents a chronic process of wound-healing that activates hyperproliferative activites resulting in BPH nodules formation. Elevated levels of TNF-α, IL-6 and CRP in the current study implies the activation of the cytokine cascade system and the multifactorial outcome which includes BPH development. However, the amelioration of TP induced BPH suggests that *N biserrata* possesses anti-inflammatory activity.

One mechanism of BPH development is oxidative damage to the prostrate tissues. Several studies have corroborated this fact. The pathogenesis of prostatic hyperplasia and prostate cancer is closely related to the state of oxidative stress in prostrate tissue [32, 33]. MDA is the end product of lipid peroxidation, a synergistic process directly linked with free radical attack. Damage to the lipid bilayer membrane of the prostrate tissues will inadvertently increase the concentration of this metabolite. The present study reveals that the concentration of tissue level of MDA was grossly increased in artificially induced cases of BPH. Likewise, the activities of the antioxidant enzyme system comprising of CAT, GPx and SOD were lowered as witnessed in the testosterone propionate only treated negative control groups. Excessive free radicals generated under oxidative stress can inhibit the activities of SOD, CAT, and GPx, further aggravating the level of oxidative stress [34, 35]. In response to hypoxia, prostrate stromal cells increase the secretion of some growth factors, such as VEGF, thus stimulating an increase in the vascular endothelial cell proliferation [36]. The ameliorative effect of the plant extract in BPH management could be due to the antioxidative potential of Nephrolepis biserrata [18]. The phytochemical composition points strongly to the anti-inflammatory and the anti-oxidative properties evidenced by *N.biserrata* in the current study. For instance, both flavonoids and saponins are well reported for their antioxidative potential as well as their activities in regulating hormonal levels.

5. Conclusion

Our study suggests that the aqueous extract of *Nephrolepis biserrata* possesses anti-BPH activity as proved by both the anti-inflammatory property and free radical scavenging action evidenced in this report. The potential mechanisms could be the down regulation of the activity of enzymatic systems particularly 5 α-reductase. The chemical composition of the plant could be responsible for these actions. Identified in the plant were the medicinally useful agents- alkaloids, flavonoids, saponins and phenolic compounds.

Article Information

Human and Animal Rights The University of Port Harcourt ethical committee (UPH/CEREMAD/REC/MM98/048) approved the study and the procedure closely compiled with the arrive recommendations as outlined in the 1996 revision of NH publications volume 25 no 28.

Contributions of Authors The study was conceptualized by Eruotor Harrison. Designed by Peters Dikioye and Monago- Ighorodje Comfort. Implementation and experimental assay were managed by Eruotor Harrison. Statistical analysis was done by Asiwe Nicholas and the first draft of the manuscript by Ezendiokwere Emmanuel.

Data Accessibility Statement This manuscript includes all of the study's data.

Funding The study was entirely self-funded by the authors.

Declaration of Competing Interest The authors affirm that they have no conflict of interest.

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